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MOSAIC

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JULIUS MATZ

A DISSERTATION SUBMITTED TO THE BOARD OF UNIVERSITY
STUDIES OF THE JOHNS HOPKINS UNIVERSITY IN
CONFORMITY WITH THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

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ARTIFICIAL TRANSMISSION OF SUGARCANE MOSAIC¹

By JULIUS MATZ²

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INTRODUCTION

Sugarcane mosaic is a disease caused by a conceivable but as yet undistinguishable virus, which has been recognized during the last decade as affecting sugarcane (*Saccharum officinarum* L.) to some extent in almost every region where sugarcane is grown commercially. For a long time investigators failed in their efforts to infect plants experimentally with the virus of this mosaic disease, and the question arose as to whether it should be considered infectious or whether the symptoms observed in sugarcane might be caused by inherent degeneration or by a nonparasitic disease. The truly infectious nature of sugarcane mosaic was firmly established about 10 years ago, but many problems still remain unsolved.

Concerning the character of the virus in the extracted plant juice, the general belief has been that if manipulated in the presence of air it would wholly, or at least largely, lose its infectiousness, particularly if inoculations were not made immediately after extraction. This belief, which was not thoroughly tested, became a serious handicap in the study of the virus. This paper presents a procedure developed during the past year for the artificial transmission of the mosaic disease, which offers not only considerable freedom in the manipulation of the extracted material but also permits the use of the virus-containing extract for a considerable time after storage. The results here recorded show that exposure to the air does not necessarily impair, under all conditions, the disease-producing properties of the extract of diseased tissues. The studies were begun in the autumn of 1929 at Johns Hopkins University and have been continued in the Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture.

HISTORICAL REVIEW

The disease now known as sugarcane mosaic has been the subject of investigations and reports by various workers, first in Java (31),³ later in Hawaii (25), and more recently in the West Indies (11, 16, 30, 33, 34, 35, 36, 37), the United States of America (6), Argentina (17), Natal (27), and India (29). In 1892 Van Musschenbroek (31) gave a description of the disease under the symptomatic name "gele strepenziekte," or yellow-strike disease, and in 1893 there appeared a multicolor reproduction showing a portion of a sugarcane leaf affected

¹ Received for publication Dec. 19, 1932; issued June, 1933. Data herein presented were included in a thesis submitted to the faculty of the Graduate School of the Johns Hopkins University, June, 1932, in partial fulfillment of the requirements for the degree of doctor of philosophy.

² Acknowledgment is made to Dr. E. W. Brandes, principal pathologist in charge, Division of Sugar Plant Investigations, for his contribution of many observations and much constructive criticism, and to Prof. Burton E. Livingston, of the Johns Hopkins University, for his help in the presentation of the results.

³ Reference is made by number (italic) to Literature Cited, p. 837.

with the disease, together with a note on its occurrence, by Arendsen Hein (3). Since 1919 the disease has been recorded (6, 7, 8, 21, 39) as affecting not only sugarcane and other species of *Saccharum* but also maize (*Zea mays* L.); sorgo (*Sorghum vulgare* Pers.) and other species of *Sorghum*; millet (*Pennisetum glaucum* (L.) R. Br.); and the wild grasses *Digitaria sanguinalis* (L.) Scop., *Paspalum boscianum* Flügge, *Setaria lutescens* (Weigel) Hubb., *S. magna* Griseb., *Panicum dichotomiflorum* Michx., *Eleusine indica* (L.) Gaertn., *Echinochloa crusgalli* (L.) Beauv., *E. colonum* (L.) Link, and *Brachiaria extensa* Chase.

In 1903 Kamerling (19), working in Java, stated that he had transmitted the disease to healthy sugarcane by inoculation with juice expressed from mosaic-diseased sugarcane. His inoculations were made by injection. Although he did not specify the kind of instrument used, it was presumably a hollow-needle syringe, since he stated that he had followed the technic employed by Beijerinck (4) in transmitting tobacco mosaic to healthy tobacco plants, and Beijerinck stated that he used a hollow-needle syringe ("Pravaz'schen Spritze"). Kamerling, regarding this sugarcane disease as infectious, placed it in the same class with the well-known mosaic disease of tobacco. His tests, however, do not appear to have fully justified such an unqualified conclusion, for some of his uninoculated and apparently healthy plants contracted the disease in the course of his experimentation. He admitted the possibility that the disease might have been transmitted in a very slight degree by air, but he expressed the conviction that at least some of the plants which he inoculated with diseased juice contracted the disease directly because of his inoculation. On the other hand, Van der Stok (38) in 1907, Kobus (20) in 1908, and Wilbrink and Ledeboer (42) in 1910—all of whom, like Kamerling, studied the disease in Java—were unable to infect healthy sugarcane plants by any of the methods they tried, including the procedure described by Kamerling.

In 1917 Stevenson (35, 36) reported the results of a study of sugarcane mosaic in Puerto Rico, referring to it as the "new disease," "the mottling disease," "an epiphytotic of cane disease," etc. It was not until 1919, however, that he identified the disease as it occurred in Puerto Rico with the gele strepenziekte, or yellow-stripe disease, described by others. Working thus independently he suspected the disease to be due to an infectious principle (37) and carried out an extensive series of inoculation tests, not only employing expressed juice from diseased cane but also inserting diseased cane tissue in incisions made in healthy plants; but he did not succeed even once in causing the disease symptoms to appear in the inoculated plants.

Stevenson's paper of 1917 (35) aroused the interest of other investigators, particularly H. L. Lyon (as reported by Colón (14)), of the Hawaiian Sugar Planters' Association, who regarded this disease as identical with the disease known as gele strepenziekte, or yellow-stripe disease, in Java, Hawaii, and elsewhere. The yellow-stripe disease had been studied by Lyon in Hawaii from 1911 to 1914 (25). In 1921 (26) he reported that in open-field experiments he had observed it in some of the plants which he had inoculated with expressed juice. Similar tests in the greenhouse failed, however, to produce the disease.

The identity of the disease causing mottling of sugarcane in Puerto Rico with the gele strepenziekte of Java and the yellow-stripe disease

of Hawaii and other regions was finally recognized by the investigators of this subject in Puerto Rico (6, 14, 16, 30, 37), and the infectious nature of the disease was generally accepted by them. Adequate evidence of its being infectious was still lacking, however; the experimental evidence was meager and not free from the possibility of error; and nothing was known regarding field dissemination.

In 1920 Brandes (7) published the outstanding discovery that sugarcane mosaic is transmitted naturally by *Aphis maidis* Fitch. Additional evidence showing the infectious nature of this mosaic and the rôle played by *Aphis maidis* in its dissemination was presented by Bruner (11) in Cuba, by Kunkel (22) in Hawaii, by Ledebøer (24) and Wilbrink (40) in Java, by Chardon and Veve (12, 13) in Puerto Rico, and by Fawcett (17) in Argentina.

Early in 1920, Brandes (7) succeeded in transmitting the mosaic disease directly from diseased to healthy sugarcane without the aid of *Aphis maidis*. By means of a glass hollow-needle syringe, he injected into the growing points of healthy sugarcane plants 0.5 cc of sap pressed from ground tissue of young joints of mosaic-diseased sugarcane. After an interval of a little more than one month, infection had appeared in 8 out of 10 plants inoculated with sap expressed under a cover of purified mineral oil, but only 2 out of 10 inoculated plants contracted the disease when the sap employed was obtained without any protective covering against exposure to air. It is important to note, however, that in both instances the inoculating material was used, as the author states, "immediately after being prepared."⁴

Brandes and Klaphaak (10) regarded lapse of time and exposure to air as the chief causes of the failure of expressed virus-containing juice to produce the disease. They say (10, p. 251)—

Virus capable of causing infection when used immediately after being expressed from diseased stalks was found in one experiment to be without effect when injected 24 hours later.⁵ The virus of grass mosaic is less stable or more sensitive to the influence of its environment than that of many other similar diseases, notably the tobacco mosaic. In these experiments it has been found very refractory and difficult of physical manipulation or chemical treatment without loss of virulence.

They also remark (10, p. 248): "Sometimes whole series of inoculations including known susceptible control plants failed, owing to unknown causes." These authors continued to use a cover of mineral oil over their inoculum, and they succeeded in transmitting the disease directly from cane to cane and from cane to sorgo, millet, and *Digitaria sanguinalis*. Thus it was definitely established not only that sugarcane mosaic is really infectious and transmissible to healthy plants of sugarcane and some related forms by the vector *Aphis maidis*, but also that it may be artificially transmitted by means of hollow-needle inoculation with freshly extracted sap from diseased plants suitably protected from the air.

Doolittle (15) found that juices of mosaic cucurbits retained the power of infection for only a short period after their extraction and usually lost their virulence within 24 to 48 hours. Dried material

⁴ In this connection Brandes (7, p. 132) credits F. S. Earle with calling attention, in a then unpublished paper, to a method of inoculating with juice expressed under oil to hinder possible oxidation. Bruner (11), however, states it as his understanding that the procedure of extracting the cane juice under mineral oil to avoid oxidation was originally suggested by E. D. Colón and F. A. Lopez Dominguez, of the insular experiment station of Puerto Rico, and that this procedure was employed later by Professor Earle.

⁵ This was not intended to imply that the virus would lose its virulence at the end of 24 hours under all conditions, as Doctor Brandes, since his first experiments in 1920, had been storing away samples of extract of sugarcane mosaic for the purpose of testing in the future the effect of age on retention of virulence.

likewise suffered a rapid loss of the infectious power. Preservatives to stop fermentation did not serve to prolong the period of activation in extracted infected juice, and low temperature had only a slight effect in prolonging the power of infection. Henderson and Wingard (18) report that the tobacco ring-spot virus is inactivated in 12 to 24 hours at ordinary room temperature and that they never succeeded in getting infection from dry material, no matter how fresh it happened to be; on the other hand, they found that the virus may retain its virulence for at least 22 months when kept at a temperature of -18°C .

Allard (1, 2) found that the tobacco-mosaic virus was still infectious after it had been bottled for four months. He later found (2, p. 636) that at the end of 231 days similarly kept extract of tobacco-mosaic virus was still highly infectious.

Earle (16, p. 17) states that—

oxidation might affect the vitality of the mosaic virus, and that a sucking insect flying from a diseased to a healthy plant and again feeding might regurgitate a minute quantity of the diseased juice without having exposed it to the air.

With juice pressed from diseased canes, without oil covering, he inoculated 7 canes by means of a hypodermic needle thrust into the leaf spindle above the terminal bud. After about 6 weeks he observed 2 infected plants, whereas from 10 inoculations made with oil-protected diseased juice he observed 5 infected plants after about 4 weeks. Seven other plants, inoculated in the midribs of young leaves, showed no symptoms of the disease. On the day after the tests with oil-protected juice some of the juice was used in 3 additional cane plants, but no positive infection developed. Because of the imperfectly known technic of inoculation and the utter lack of understanding of host response and reaction, little could be said in favor of one procedure over the other, particularly when there seemed to be a lack of uniformity in the results, even with the method that had been successful in one instance.

At about the same time the writer (30) obtained 2 mosaic infections among 5 cane plants that had been inoculated with air-exposed infected juice. In another similar experiment he obtained 2 infections out of 20 inoculations. In both tests the period between inoculation and the appearance of the first mosaic symptoms was about 14 days. The tests of both Earle and the writer, however, unlike those of Brandes, were performed under conditions that did not preclude the possibility of natural infection by aphids.

Following the studies of Brandes, Earle, and the writer, Bruner (11) attempted to transmit sugarcane mosaic, sometimes through the agency of *Aphis maidis* and sometimes without employing the insect vector; he also considered the possibility that exposure to air might have an inhibiting effect upon the virus in pressed-out sugarcane sap. To reduce the air exposure of the sap intended for inoculation he superimposed a mosaic-infected leaf upon a healthy leaf and, holding the two in close contact by finger pressure, he thrust a fine hypodermic needle rapidly through the infected leaf into the healthy one. In this manner he inoculated a tender leaf, a mature leaf, and an old leaf on each of 100 shoots comprising altogether 23 cane stools of the variety *Cristalina*. The experiment was made November 6, 1920; and in March, 1921, only eight shoots in 3 stools showed infection. Bruner believed that the small percentage of

infection in this experiment might have been due to the fact that his inoculated plants were well advanced in development and the rate of growth was slow. In another experiment (his No. 8) Bruner injected infected juice that had been extracted under a cover of oil into 15 sugarcane plants of the variety Cavengerie, while 15 other plants of the same variety were similarly injected with juice extracted in the air. The operations were performed rapidly and in each series only one minute intervened between extraction and inoculation. In each of the lots 3 plants became infected within about 8 weeks from the time of inoculation. Bruner (11, p. 21) states (translation):

We can conclude that the diseased cane juice extracted without any provision against oxidation will reproduce the mosaic just as the juice extracted under a protective cover of oil if there is no delay in making the inoculation after extracting the juice.

In 1920 Brandes (7) obtained no infection of cane plants of the Lahaina variety when juice from infected leaves, prepared without any oil protection, was rubbed with the fingers into either unbroken or needle-scarified surfaces of young leaves; nor did any infection result when inoculation was accomplished by scarifying the young leaf cells with a sharp needle dipped in the air-exposed mosaic-infected juice. Only 1 infection resulted from 10 inoculations made by numerous needle pricks with the same air-exposed juice. In other tests, where the inoculation methods just mentioned were employed except that the diseased juice was pressed from young stems under mineral oil, there were no infections. When a hypodermic syringe was used, 8 out of 10 inoculations made with oil-protected inoculum were successful, whereas only 2 out of 10 inoculations made with unprotected inoculum were successful. Using absorbent cotton wet with juice that had been pressed (without oil) from leaves and upper joints of diseased Lahaina sugarcane, Kunkel (23) rubbed the inoculum into wounded leaves of healthy Striped-Tip sugarcane plants. He performed 3 experiments, each with 6 plants, but reported no infections except from the third experiment, after a lapse of about 3 months, when 5 of the plants showed mosaic symptoms. From 2 to 3 weeks usually intervenes between the date of inoculation and the first appearance of symptoms of this disease.

McRae and Subramaniam (29), using a needle, pricked juice from freshly crushed mosaic leaves into the leaf sheaths and stems of sugarcane, maize, and sorghum. They reported many successful inoculations, but the proportion of infection to inoculation was highly variable; for instance, out of 16 shoots of the cane variety Coimbatore 213, inoculated with juice from diseased Red Mauritius cane, all showed the disease; but out of 30 shoots of Coimbatore 213 inoculated with juice from Coimbatore 205 only 2 developed the disease. While this variability may perhaps have been due in some degree to differences in host relationships and possible attenuation of the virus in some varieties, it seems probable that the method by which the inoculum was secured and introduced into the healthy tissue was often at fault.

Sein (32), using a fine needle, pricked freshly expressed diseased juice (without any oil covering) into the tender parts of the tightly rolled young leaves of cane shoots and obtained more than 50 successful infections in one experiment with 100 inoculated plants. Subsequently he abandoned the use of extracted sap and adopted the

principles of Bruner's (11) method; a band of expanded mosaic leaf was held tightly wrapped around the stripped cylinder of young, tender leaves of the healthy plant and the pin point was repeatedly thrust through the diseased tissue into the healthy leaves. Out of 100 shoots inoculated in the open field by this method, 94 per cent showed infection. Sein thought that his very fine needle, thrust rapidly through the previously unbroken band of diseased leaf into the still tightly rolled cylinder of healthy young leaves, carried diseased material almost directly into healthy tissue without any considerable exposure of the material to the air. It is apparent, however, that if exposure to air is really detrimental to this virus, the air between the two rather rough leaf surfaces may be sufficient to affect the virulence of the minute quantity of infected juice carried by the needle, no matter how firmly the band of diseased leaf is held by the fingers against the leaf cylinder. The advantage of using a very fine needle, as recommended by Sein, would come from the fact that the very small stab lesion resulting from the puncture would cause very little injury, and that the virus would thus be introduced directly into an environment of living cells.

A study of these recent papers on the artificial transmission of sugarcane mosaic makes it evident that their authors have generally thought that this virus (or at any rate the expressed juice from diseased tissues) becomes greatly weakened or loses its capacity for producing mosaic symptoms if the inoculum is not used very soon after it is prepared, and especially if air has access to it during preparation or in the process of inoculation.

It appears, though it has not been exactly thus stated, that the disease-producing capacity of the virus of sugarcane mosaic depends on its being in intimate and practically uninterrupted association with the living cell contents of its host. No one has as yet recorded the occurrence of infection in healthy sugarcane plants from the application of decayed or dried material from infected sugarcane plants or from air-exposed extract of diseased cane tissue when the extract has been kept a long time at ordinary room temperatures. It is commonly supposed that there are marked differences in susceptibility to visible infection, not only among different varieties of cane at any given stage of growth but also among the several parts or regions of an individual plant. The inoculum from diseased plants has generally been applied to the growing point of the cane plant or the youngest leaves, and it seems to be an accepted conclusion that cane roots are not at all receptive to the introduction of the disease. The capacity of the virus to produce the symptoms of sugarcane mosaic seems to depend on a narrow range of external conditions as well as on the varying internal reactions of the host.⁶

EXPERIMENTAL METHODS

The inoculum used in the experiments reported in this paper was obtained from young mosaic-infected suckers which sprang up from time to time from the bases of three mosaic-infected fully grown stools of the sugarcane variety P. O. J.⁷ 234. After the mature leaves had been discarded the remaining leafy portions, including the false

⁶ The inoculation method employed by McKinney (28) in his work with wheat and rye was tested, but in no instance did the inoculated sugarcane show infection.

⁷ Proefstation Oost Java.

stems, of about 10 shoots were cut into $\frac{1}{2}$ -inch lengths and ground in a meat chopper, slowly, in order to avoid overheating the material. The sharply grooved nut-butter-cutter disk furnished with the chopper was employed. All apparatus used had been sterilized by boiling in hot water. The ground material was received from the chopper in a 20-ml porcelain evaporating dish, most of the liquid being then squeezed out by hand through cheesecloth. The nearly dry pulp was discarded and the green filtrate, which contained much material in suspension, including whole and broken chloroplasts, was stored in vials or flasks, without special precautions of any kind except, in some instances, in regard to temperature.

When a cane shoot was to be inoculated some of this liquid was transferred by means of a pipette to the wedge-shaped opening between the youngest expanded leaf blade and the next younger leaf on the same side, which was still rolled. The blade of the youngest expanded leaf bends sharply away from the vertical axis of the plant, and its base partly clasps the false stem or leaf spindle, thus forming a sort of cup or reservoir that retains the liquid, while a small area of the outer surface of the most recently exposed but still rolled leaf is covered by the liquid. The relations of these two leaves and the expressed juice held between them are shown in Figure 1. A fine needle point (usually No. 216, Special Minuten Nadeln), set into a glass rod which served as a handle, was next passed horizontally, or somewhat obliquely downward, through the liquid and into the submerged area of the still rolled leaf. In addition to the direct downward punctures, several vertical cuts with the needle were made through the leaf tissue in order to allow the contact of the virus with the now severed fine transverse connections of the vascular bundles. This stabbing operation was repeated rapidly about 5 or 6 times, with gentle pressure; each needle thrust formed a new puncture, but all punctures were close together and were covered by the juice in the reservoir. This method of inoculation was found to be suitable for young sorghum and *Digitaria* plants as well as for sugarcane. Inoculations were usually made on a cloudy afternoon, when the light intensity was weak and evaporation was slow.

The inoculated plants stood for many weeks in pots of soil in an ordinary greenhouse, at a temperature varying from about 25° to 30° C., and were frequently inspected. Each inoculated culture had a green label showing how and when inoculation was performed. When mosaic symptoms appeared a red label was attached, bearing the date of the first appearance of the disease. Subsequent notes were added as the malady progressed.

The greenhouse was screened, and no individuals of *Aphis maidis*, the natural transmitter of the disease, were observed in the compartments used or in adjacent ones. As has been said, no special precautions were taken to protect the expressed juice from the air. Nor were any pains taken to use the extract immediately, although many inoculations were made on the same day as that on which the extract was prepared—usually after about 2 to 4 hours. When inoculations were not to be made until the day after the extraction or later, the inoculum was stored in open beakers or vials in an electric refrigerator at a temperature of about 4° C. At this temperature, as well as at higher ones, considerable development of bacteria and other micro-organisms was observed after 24 hours, and it was suspected that these

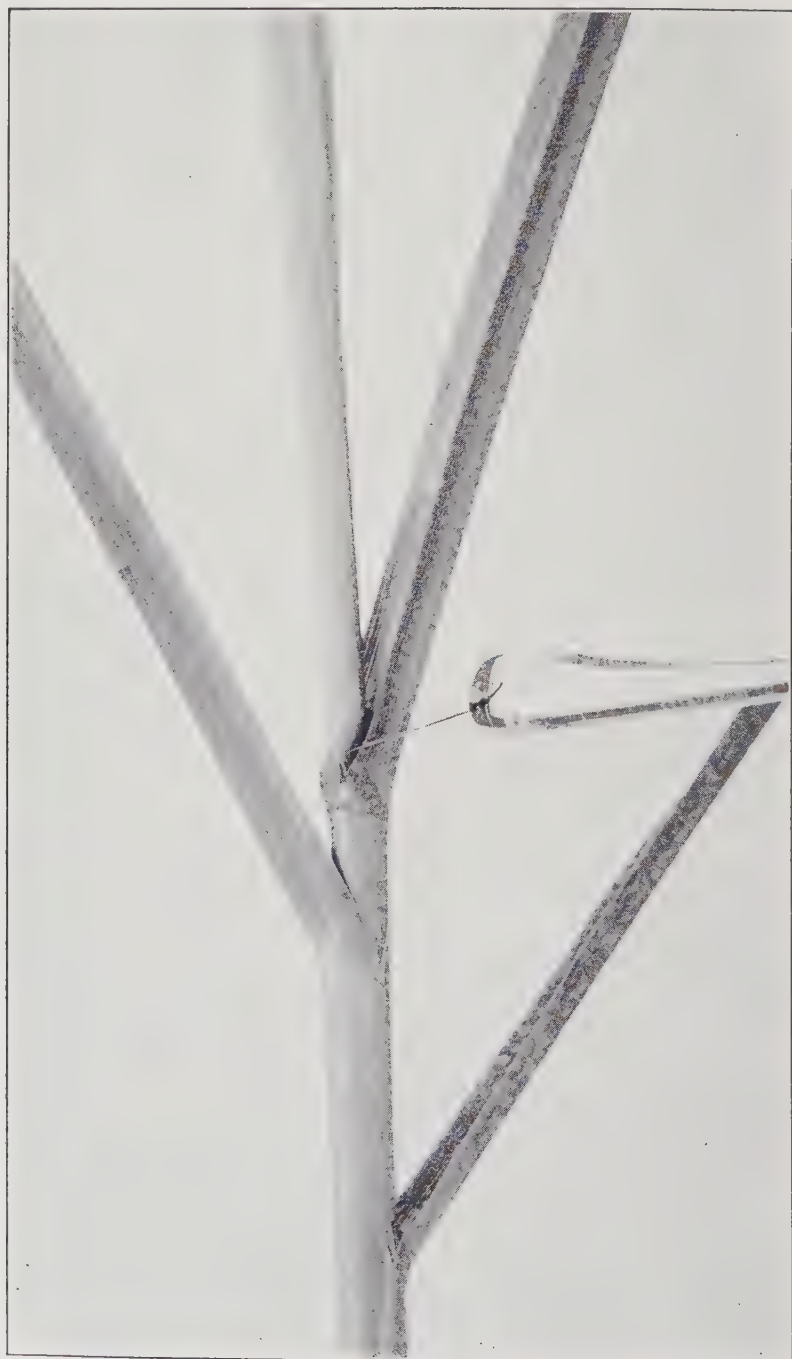


FIGURE 1.—Inoculation of a sugarcane shoot with extracted juice of a mosaic-infected plant. A drop of infected liquid is placed at the wedge-shaped opening between the youngest expanded leaf blade and the next younger, still rolled leaf. The fine needle point is passed through the liquid and into the liquid-covered area of the rolled leaf.

might partly account for some inactivation of the stored extract. Tests in which stored extract was kept at a temperature below the freezing point of water were therefore made. For these tests several somewhat different procedures were tried. (1) Freshly expressed juice from infected cane was kept in open vials in the freezing compartment of an electric refrigerator from within 1 hour after the crushing of the diseased material until the inoculum was used. The temperature of this compartment was $-6^{\circ}\text{C}.$, and the liquid became solidly frozen within 24 hours, being thawed at room temperature just before use. (2) Fresh diseased extract in similar open vials was frozen solid within 20 minutes after extraction, by means of blocks of solid carbon dioxide placed alongside in a small, suitably insulated box. The vials of frozen inoculum were then transferred to the refrigerator compartment just mentioned, where the material remained frozen, at about -6° until thawed for use. (3) Fresh juice from mosaic sugarcane was first stored for 6 days at 4° ; it was then transferred to the freezing compartment of the refrigerator (-6°) and stored there until thawed for use.

EXPERIMENTAL DATA

INOCULATION TESTS

Data for several hundred inoculation tests made in the spring and summer of 1931 are presented in Table 1. In all these the inoculum employed was juice expressed from mosaic-diseased sugarcane of the variety P. O. J. 234, and the method of inoculation was usually that described in the preceding section. It is, of course, understood that a single inoculation of this kind includes several separate needle punctures, all close together. This type of inoculation is herein designated as the "regular" method. In a few instances other methods of inoculation were used. Tests were made on the Black Amber variety of sorgo and on six mosaic-susceptible varieties of sugarcane, namely: Louisiana Purple, P. O. J. 36-M⁸, D⁹-74, P. O. J. 234, Cristalina, and B. H.¹⁰ 10/12. In each experiment a young, vigorously growing, and apparently healthy plant 1 to 2 feet in height was employed for each inoculation. The results of these tests are shown in Table 1.

Specific records of control plants are omitted, since such records would constitute merely a series of repetitions. In each test, however, control plants as nearly like the inoculated plants as possible were used. The controls received the same treatment as the inoculated plants except that they were not inoculated. The number of controls was usually the same as the number of inoculated plants, but in some instances it was much larger. Several hundred healthy sugarcane plants growing in the greenhouse were continually under observation until March, 1932, and these served as additional, though undesignated, controls. None of the uninoculated plants, whether designated as controls or not, showed any mosaic symptom during the period of observation (from April, 1931, to March, 1932). As the records show, however, inoculation did not always produce infection.

⁸ Mingka selection of P.O.J. 36.

⁹ Demerara.

¹⁰ Barbados hybrid.

TABLE 1.—Results of inoculating Black Amber sorgo and six susceptible varieties of sugarcane with mosaic virus under various conditions in 1932

Variety	Experiment No.	Inoculum		Inoculations			Infections observed		
		Age	Storage temperature	Date	Number	Method	Date	Number	Total
Sorgo (Black Amber)-----	1	Hours 2-4	° C. (a)	Apr. 27	12	(b)	May 11 May 23 May 26 do. 1 May 28 May 29 June 4	1 4 3 1 2 1 4	8
	2	24	4	Apr. 28	12	(c)	May 11 June 3	1 1	2
	3	24	4	do.-----	12	(d)	June 3	1	1
	4	2-4	(e)	May 27	6	(e)	June 12	0	0
	5	2-4	(a)	do.-----	6	(e)	June 18	2	3
Sugarcane:	6	24	4	Apr. 28	6	(e)	May 11 May 15 May 24 May 25 May 28	1 1 2 1 1	5
	7	24	4	May 15	13	(e)	June 1 June 8 June 17 May 28	1 1 4 1	7
	8	24	4	do.-----	12	(f)	June 8 June 17 June 17	1 1 1	3
	9	2-4	(a)	May 25	11	(e)	June 7	5	6
	10	2-4	(a)	do.-----	10	(e)	June 9	1	0
Louisiana Purple-----	11	Days 11	4	do.-----	5	(e)	June 8	1	1
	12	10	4	June 23	11	(e)	July 24	3	3
	13	Hours 2-4	(a)	May 27	6	(e)	June 18 June 22 July 17	4 1 1	5
	14	Days 26	4	June 19	6	(e)	July 17 July 22 July 23 July 31	1 1 1 1	3
	15	Hours 2-4	(a)	July 20	30	(e)	Aug. 5 Aug. 6 Aug. 7 Aug. 9 Aug. 11 Aug. 18 Aug. 27 Sept. 22	5 5 1 2 2 2 1 2	20
	16	24	4	July 21	24	(e)	Aug. 5 Aug. 7 Aug. 8 Aug. 19	1 1 1 1	4
	17	Days 14	4	do.-----	30	(e)	Aug. 5 Aug. 7 Aug. 11 Oct. 6	2 3 1 3	6
	18	28	g-6	Sept. 17	10	(e)	Oct. 8 Oct. 19 Oct. 1	1 1 1	5
	19	22	h-6	do.-----	10	(e)	Oct. 5 Oct. 6 Oct. 7 Oct. 8 Oct. 20	2 1 1 1 1	7
	20	28	i-6	do.-----	10	(e)	Oct. 5	1	1
P. O. J. 36-M-----	16	24	4	July 21	24	(e)	Aug. 5 Aug. 7 Aug. 8 Aug. 19	1 1 1 1	4
	17	Days 14	4	do.-----	30	(e)	Aug. 5 Aug. 7 Aug. 11 Oct. 6	2 3 1 3	6
P. O. J. 36-M-----	18	28	g-6	Sept. 17	10	(e)	Oct. 8 Oct. 19 Oct. 1	1 1 1	5
	19	22	h-6	do.-----	10	(e)	Oct. 5 Oct. 6 Oct. 7 Oct. 8 Oct. 20	2 1 1 1 1	7
P. O. J. 36-M-----	20	28	i-6	do.-----	10	(e)	Oct. 5	1	1

^a The inoculum was stored at room temperature.

^b The plants were inoculated by rubbing the extract into the young leaves by means of a glass rod.

^c The regular method of inoculation was employed (p. 827).

^d The plants were inoculated by injecting the extract into a rolled leaf by means of a hypodermic syringe.

^e The regular method of inoculation was employed, but the leaf tissue was not punctured.

^f The regular method of inoculation was employed except that a coarse needle was used.

^g The extract was frozen within 20 minutes after being prepared, previous to storage.

^h In storage the extract became frozen within about 24 hours after preparation.

ⁱ The extract was stored for 6 days at 4° C., then frozen and stored at -6°.

TABLE 1.—Results of inoculating Black Amber sorgo and six susceptible varieties of sugarcane with mosaic virus under various conditions in 1932—Continued

Variety	Experiment No.	Inoculum		Inoculations			Infections observed		
		Age	Storage temperature	Date	Number	Method	Date	Number	Total
D-74		Hours	° C.						
	21	24	4	June 9	16	(c)	June 22 June 24	1 2	3
	22	24	4	June 11	8	(c)	June 29 July 3	1 1	2
	23	Hours 2-4	(a)	July 2	6	(c)	Aug. 5	5	5
	24	Days 28	a -6	Sept. 17	10	(c)	Oct. 5 Oct. 9 Oct. 12 Oct. 20	3 1 1 1	6
	25	22	h -6	do	10	(c)	Oct. 2 Oct. 7	1 1	2
	26	28	i -6	do	10	(c)		0	0
	27	28	4	June 22	12	(c)	July 6 July 13 July 15	1 1 1	3
	28	28	4	June 24	11	(c)	July 20	1	1
	29	28	a -6	Sept. 17	10	(c)	Oct. 1 Oct. 5	2 1	3
P. O. J. 234	30	22	h -6	do	10	(c)	Oct. 1 Oct. 5	1 2	3
Cristalina	31	28	i -6	do	10	(c)		0	0
	32	30	4	June 27	6	(c)	July 11 Oct. 5	2 1	1
B. H. 10/12	33	28	a -6	Sept. 17	10	(c)	Oct. 5 Oct. 6 Oct. 9	1 1 1	4
	34	22	h -6	do	10	(c)	Oct. 1 Oct. 5	1 2	5
	35	28	i -6	do	10	(c)	Oct. 9	2	0

a The inoculum was stored at room temperature.

c The regular method of inoculation was employed (p. 827).

e The extract was frozen within 20 minutes after being prepared, previous to storage.

h In storage the extract became frozen within about 24 hours after preparation.

i The extract was stored for 6 days at 4° C., then frozen and stored at -6°.

RELATIVE EFFECTIVENESS OF DIFFERENT METHODS OF INOCULATION

Experiment 1, Table 1, shows that sorgo may be successfully inoculated by merely rubbing the extract into the leaves without puncturing the tissue; out of 12 tests, 8 were successful. This rubbing method is not usually so efficient in transmitting the disease, however. Experiments 2 and 3, also with sorgo, show the relatively low degree of efficiency of inoculation by means of a hypodermic syringe as compared with inoculation by means of a drop of inoculum and several fine-needle punctures (regular method). Out of 12 tests, the syringe method gave only 2 infections; the regular method gave 8.

Experiments 4 and 5, also with sorgo, confirm the frequently repeated observation that merely placing inoculum on the surface of the young leaf, without puncturing, is not a successful method of inoculation. In each of these cases there were 6 tests; the regular method gave 3 infections; the same procedure without puncturing gave none. Experiments 9 and 10, with Louisiana Purple sugarcane, show similar results; the regular method gave 6 infections out of 11 inoculations, whereas 10 similar inoculations without puncturing resulted in no infection.

Experiments 7 and 8, with Louisiana Purple sugarcane, indicate that where the regular method of inoculation is employed a very fine needle gives a higher percentage of successful infections than does a coarse dissecting needle. In these two experiments the use of a very fine needle resulted in 7 infections out of a possible 13, whereas when a coarse dissecting needle was employed there were only 3 infections out of a possible 12.

INFLUENCE OF TEMPERATURE AND AGING ON INFECTIOUSNESS OF INOCULUM

Because of the preliminary nature of the study reported in this paper, the data available for any single variety of plant are not sufficiently extensive to warrant quantitative comparison of the several varieties tested. It is obvious, however, that when inoculation was made in the regular manner, all the varieties were capable of infection.

The relations between the result of inoculation and the temperature and period of storage of the inoculum may be shown if the whole series of experiments in which the regular method of inoculation was employed is divided into categories according to the preinoculation treatment given to the inoculum, without reference to either plant variety or number of repetitions. Such a classification is shown in Table 2, which comprises all the experiments listed in Table 1 except Nos. 1, 3, 4, and 8, in which the regular method was not employed. Since there is no evidence that date of inoculation or season of the year have influenced the results of these experiments, the dates of the experiments, as given in Table 1, are not repeated in Table 2. The percentages of infection for the various groups, given in the last column of Table 2, indicate approximately the corresponding relative effects of the various treatments on the infectiousness of the mosaic virus.

The mean percentages of infection for groups 1 to 4 indicate that the virulence or infectiousness of the extract used as inoculum tended to decrease with longer storage at a temperature as low as 4° C. The tests of group 1 (with nearly fresh extract) show a mean percentage of 66, those of group 2 (with extract that had been stored about a day at 4°) show a corresponding percentage of 44, and those of group 3 and of group 4 (with extracts that had been stored at 4° for about a fortnight and for about a month, respectively) show corresponding values of only 22 and 21. Infectiousness appears to have been reduced, however, only by about two-thirds (from 66 to 21 per cent) as a result of the longest storage at 4°. About two weeks of storage at 4° appears to have resulted in as much reduction as was shown when the extract had been stored nearly a month; the mean infection percentages for groups 3 and 4 are not significantly different. These results show that expressed juice of mosaic-infected sugarcane stored in open beakers or vials for several weeks at a temperature somewhat above the freezing point of water did not completely lose its infectiousness. Such storage, however, even for a single day, produced a marked reduction in the mean value of the infection percentage.

TABLE 2. —*Relative infectiousness of similar mosaic extracts (from infected sugarcane P. O. J. 234) variously treated prior to their use in making inoculations*

Group No.	Treatment of inoculum	Experiment No.	Plant inoculated	Inoculations	Infections		
				Number	Number	Per cent	
1	Extract stored only about 2 to 4 hours at room temperature.	5	Sorgo	6	3	50	
		9	Louisiana Purple cane	11	6	55	
		13	P. O. J. 36-M cane	6	5	83	
		15	do.	30	20	67	
		23	D-74 cane	6	5	83	
Total			59	39	66		
2	Extract stored about 1 day at 4° C.	2	Sorgo	12	8	67	
		6	Louisiana Purple cane	6	5	83	
		7	do.	13	7	54	
		16	P. O. J. 36-M cane	24	4	17	
Total			55	24	44		
3	Extract stored 10 to 14 days at 4° C.	11	Louisiana Purple cane	5	1	20	
		12	do.	11	3	27	
		17	P. O. J. 36-M cane	30	6	20	
Total			46	10	22		
4	Extract stored 25 to 28 days at 4° C.	14	P. O. J. 36-M cane	5	3	60	
		21	D-74 cane	16	3	19	
		22	do.	11	2	18	
		27	P. O. J. 234 cane	12	3	25	
		28	do.	11	1	9	
		32	Cristalina cane	6	1	17	
Total			61	13	21		
5	Extract frozen within 20 minutes after preparation, then stored about 27 days at -6° C.	18	P. O. J. 36-M cane	10	5	50	
		24	D-74 cane	10	6	60	
		29	P. O. J. 234 cane	10	3	30	
		33	B. H. 10/12 cane	10	4	40	
Total			40	18	45		
6	Extract stored about 27 days, at -6° C., becoming frozen within first 24 hours.	19	P. O. J. 36-M cane	10	7	70	
		25	D-74 cane	10	2	20	
		30	P. O. J. 234 cane	10	3	30	
		34	B. H. 10/12 cane	10	5	50	
Total			40	17	43		
7	Extract first stored 6 days at 4° C., then frozen and stored 20 days at -6°	20	P. O. J. 36-M cane	10	1	10	
		26	D-74 cane	10	0	0	
		31	P. O. J. 234 cane	10	0	0	
		35	B. H. 10/12 cane	10	0	0	
Total			40	1	3		

To secure the highest percentage of infection it appears to be desirable to use the extracted juice while fresh. Storage in an ordinary refrigerator, however, may be expected to preserve the infectiousness of this virus for a long time, despite its apparently progressive loss of virulence.

The percentages for groups 5, 6, and 7 indicate that a storage temperature somewhat below the freezing point of water preserved the virulence of the virus much more satisfactorily than did a temperature of about 4° C. As might be expected, for freezing storage it appears to be desirable to bring the extract into the frozen condition soon after its preparation, for a 6-day storage at 4° followed by a 20-day storage at -6° gave very unsatisfactory results (group 7, with mean percentage of only 3). Nevertheless, the percentage value for group 5 (with quick freezing) is only slightly—and probably not significantly—higher than that for group 6 (without quick freezing). It

may therefore be recommended that extract of mosaic-infected sugarcane intended for inoculation tests similar to those of the present study should be prepared at a temperature as low as is feasible (with cold press and vessels) and brought to a freezing storage temperature promptly. It is important to note that extract stored for nearly a month at -6° (groups 5 and 6) gave, on an average, infection percentages (45 and 43), approximately the same as that given by similar extract stored at 4° for a single day (group 2, with a mean percentage of 44). Since it appears that the freezing of the extract did not diminish its infectiousness, it might be desirable to employ freezing of the infected tissue to aid in freeing the juice from the cells, as has frequently been done when plant tissues were to be extracted for other purposes. The infected material would thus be first frozen, then thawed, ground, and pressed, and finally stored at a freezing temperature.

Although the rather broad field explored in this preliminary study and the consequent lack of extensive series of strictly comparable data make the application of detailed numerical computations and more precise analytical methods inapplicable, yet it may be interesting to note that the highest infection percentages for individual experiments are shown for experiments 13 and 23 (group 1, with fresh extract) and for experiment 6 (group 2, with extract that had been stored about 1 day at 4° C.), each of which gave 5 infections out of 6 inoculations, or 83 per cent of successful transmission of the disease. The next lower individual percentages (67 to 70) were given by experiment 15 (group 1, with fresh extract), experiment 2 (group 2, with extract that had been stored about 1 day at 4°), and experiment 19 (group 6, with extract that had been stored about 27 days at -6°).

DISCUSSION

The causative substance or virus of the sugarcane mosaic disease, when separated from the host, seems to be much more sensitive to its surroundings than are some of the other and better known plant viruses, which may be readily and successfully transmitted by the simple operation of rubbing into the bruised tissues of an uninfected plant a minute quantity of material from crushed cells of a diseased plant.

The deterioration of the juice expressed from infected plants is probably the result of a number of factors. When cells are crushed and pressed the physical, chemical, and physiological influences normally prevailing in the living cell contents must undergo great alteration; the characteristic vital coordinations are disturbed and the activities of various catalytic agents, such as the enzymes, become either retarded or accelerated. Furthermore, the relations between the extracted juice and the atmosphere, especially those connected with the interchange of oxygen and carbon dioxide, must be very different from the corresponding relations between living cell contents and the atmosphere. Moreover, bacteria or other microorganisms may become active in the expressed juice.

The many failures that have attended attempts to transmit the sugarcane mosaic by means of expressed sap, even when freshly prepared, indicate that successful transmission may depend on some specific manner of introducing the extracted sap into the healthy plant. The tissue with which the inoculum first comes in contact must serve as a suitable and receptive medium for the continuation

of the life or activity of the infective substance, or it must at least offer a vehicle for the conduction or transfer of that substance to cells that are capable of harboring it and disseminating it through the plant. In his study of the mechanics of inoculation, Brandes (9) pointed out that *Aphis maidis*, the only known natural vector of sugarcane mosaic, apparently causes no perceptible destruction of tissue as it feeds on the leaf of maize, although the fine setae of the insect's proboscis pass through the epidermis of the leaf, and generally pass several cell layers until they penetrate the phloem. The slight surface wounding caused by the aphid seems to produce no serious injury, nor do the disease symptoms appear first at the place of puncture. As shown by the writer (30) and others, mere surface contact between healthy and diseased sugarcane plants does not bring about transmission, since leaves of healthy plants in the field in contact with leaves of diseased plants remained healthy indefinitely.

It seems clear that in order to produce infection, virus or infected juice must come into intimate contact with the inner cells. But artificial applications to interior tissues do not always lead to infection. According to Stevenson (37), Earle (16), the writer (30), and Sein (32), the application of diseased tissue or juice to knife-cut surfaces of internal stem tissue of healthy plants or cuttings did not produce the disease. Nevertheless, Bonazzi (5) reported successful inoculations through comparatively large stem wounds made with a borer, and Wilbrink (41) induced infection in healthy seed cuttings by cutting them with a knife that had been used to cut diseased cane. Brandes (9) suggested that in plants inoculated by means of aphids the phloem may be the path of transmission and that in successful artificial inoculations through mature stem tissue the infection may have been transmitted to the new shoots by way of the vascular bundles rather than through the mature and inactive parenchymatous tissue. The success or failure of inoculation experiments with this mosaic may be explained by assuming that infection is most apt to result from artificial introduction of diseased juice if the operation is so performed as to bring the inoculum into immediate contact with some special cells of the vascular bundles without undue injury either to these or to the overlying tissues.

Since this virus is systemic in the infected host plant; since the host with the virus in it continues active existence almost indefinitely, although in many cases small regions of dead or shrunken parenchyma may be observed in the stems of infected plants (30); and since the virus loses its potency in dead tissue, it follows that the virus is not only naturally adapted to the living cells of the host but that its virulence depends upon the conditions of living tissue.

When successful inoculations were made through the rolled leaves of a young cane plant the first visible symptoms of mosaic were generally observed only after two weeks or more, usually as much as 12 inches or more below the point of inoculation and in the second or third leaf, but not in the outermost and oldest leaf of the spindle into which inoculation had been made. At the same time first symptoms were often discernible in inner leaves of the spindle that had not been reached by the infecting instrument at all. The virus must have descended several inches through the first infected older leaf or leaves to their insertions, whence it seems to have passed into the bases of the younger leaves, moving upward with their growth until the symptoms

became visible as the leaves expanded. In this respect sugarcane mosaic is like other mosaic diseases. It may, therefore, be supposed that the virus is not at once active in the production of mosaic symptoms in an inoculated leaf, but that it passes downward in that leaf for a long distance without causing visible injury and produces visible symptoms only after it has moved upward into still younger leaves. This supposition appears to agree with observed facts. There is reason for believing that the path of descent may be in the phloem.

Whatever the method by which diseased sugarcane juice has previously been applied to healthy plants—whether it has been rubbed into wounded leaves, injected with a hypodermic syringe, or pricked in with a needle—no attempt apparently has been made to prevent access of air to the lesions. It is reasonable to suppose that the hydrostatic and gas pressures that prevail in the vessels and in the closed intercellular spaces of the healthy sugarcane leaf are somewhat lower than the gas pressure of the external air and of the substomatal and other gas-filled spaces that are in direct communication with the external air, especially when transpiration is proceeding rapidly. Under such conditions of pressure gradients, minute quantities of air would promptly enter any wound or incision made in the leaf, usually before the entrance of the liquid inoculum, no matter how promptly the latter might occur. Thus it may happen that at least some portions of the internal tissue surfaces suddenly exposed by a mechanical incision become covered with a thin gas layer before they are really reached by the diseased juice, and actual access of the latter to such gas-covered surfaces may be greatly retarded or even prevented. It is believed that these possibilities were largely avoided in the present experiments by first covering the region where inoculation incisions were to be made with a drop of the liquid inoculum, which remained in position as a water seal over the incisions until reduced and removed through evaporation and absorption; suction or diffusion of virus into the wounds continued to occur long after the completion of the stabbing operation. This expedient of making the needle stab through a properly placed drop of diseased juice gave added assurance that the needle point was always thoroughly wetted with the liquid as it entered the foliar tissue.

Because of turgor, the hydrostatic pressure of living cells, including the elements of the phloem, is greater than that of the sap in the vessels; it exceeds also the gas pressure of the atmosphere and of the intercellular spaces. Hence some exudation may occur from living cells that happen to be penetrated by the stabbing needle. When stabbing is performed through a drop of diseased juice the sucked-in inoculum should tend to come into immediate contact with any extruded cell contents, and the complex system of surface-tension gradients thus set up should result in convection currents that would effectively mix the two liquids. This sort of mixing action would be much more rapid than simple diffusion and might continue for a long time after the operation of stabbing had been completed.

SUMMARY AND CONCLUSIONS

In this paper the present state of knowledge concerning the mosaic disease of sugarcane is reviewed and new experimental results of inoculation tests with this mosaic are presented. A new method of

inoculation is described. In inoculation tests on healthy plants of the mosaic-susceptible sorgho variety Black Amber and on the mosaic-susceptible sugarcane varieties, the extracted juice of diseased sugarcane was used as inoculum. In making an inoculation a drop of inoculum was placed in the wedge-shaped opening between the base of the youngest expanded leaf blade and the next younger leaf, recently exposed but still tightly rolled, on the same side of the false stem or leaf spindle. Through the liquid held in this wedge-shaped reservoir a very fine needle was repeatedly thrust with gentle pressure, pricking the submerged area of the rolled leaf. The pricking or stabbing was thus done under the liquid, which was allowed to remain in place till it evaporated or was absorbed. Where fresh or properly stored juice was used, high percentages of infection generally resulted within a few weeks, although the juice was never protected from the air. Except in inoculated plants, no infection was observed at any time during the investigation either in plants serving as controls or in other plants in the greenhouse.

The results obtained indicated that expressed juice of mosaic-infected sugarcane tends increasingly to lose its infectiousness if kept in open beakers or vials for a day or more at room temperature or even at a temperature of about 4° C. This tendency was largely overcome, however, when the juice was stored in open vessels at a temperature of about -6°, at which the liquid became solidly frozen within 24 hours, and so remained until thawed at room temperature just before it was used in inoculation tests. High percentages of infection were obtained when inoculations were made (by the new or "regular" method) with juice that had been stored in the frozen condition for as long as 27 days. Infectious juice that had been kept for 6 days at a temperature of 4° and was then frozen and stored for 20 days at -6° was found to have lost most or all of its infectiousness. In the light of present knowledge it may be recommended that infected juice for inoculation experiments of the sort here reported be frozen as soon as feasible after its preparation and be kept frozen until thawed for use; and also that the grinding and pressing operations by which infected juice is prepared should be performed at a temperature as low as possible.

LITERATURE CITED

- (1) ALLARD, H. A.
1914. THE MOSAIC DISEASE OF TOBACCO. U. S. Dept. Agr. Bul. 40, 33 p., illus.
- (2) ———
1918. EFFECT OF VARIOUS SALTS, ACIDS, GERMICIDES, ETC., UPON THE INFECTIVITY OF THE VIRUS CAUSING THE MOSAIC DISEASE OF TOBACCO. Jour. Agr. Research 13:619-637.
- (3) ARENDSSEN HEIN, S. A.
1893. VRAAGBORD. Arch. Java Suikerindus. 1: [215]-216, illus.
- (4) BEIJERINCK, M. W.
1898. UEBER EIN CONTAGIUM VIVUM FLUIDUM ALS URSACHE DER FLECKEN-KRANKHEIT DER TABAKSBLÄTTER. Verhandl. K. Akad. Wetensch. Amsterdam (2 sect.) deel 6, No. 5, 21 p., illus.
- (5) BONAZZI, A.
1926. STUDY ON SUGAR CANE MOSAIC. Science (n.s.) 64:529-530.

- (6) BRANDES, E. W.
1919. THE MOSAIC DISEASE OF SUGAR CANE AND OTHER GRASSES. U. S. Dept. Agr. Bul. 829, 26 p., illus.
- (7) ———
1920. ARTIFICIAL AND INSECT TRANSMISSION OF SUGAR-CANE MOSAIC. Jour. Agr. Research 19:131-138.
- (8) ———
1920. MOSAIC DISEASE OF CORN. Jour. Agr. Research 19:517-522, illus.
- (9) ———
1923. MECHANICS OF INOCULATION WITH SUGAR-CANE MOSAIC BY INSECT VECTORS. Jour. Agr. Research 23:279-284, illus.
- (10) ——— and KLAPHAAK, P. J.
1923. CULTIVATED AND WILD HOSTS OF SUGAR-CANE OR GRASS MOSAIC. Jour. Agr. Research 24:247-262, illus.
- (11) BRUNER, S. C.
1922. SOBRE LA TRANSMISION DE LA ENFERMEDAD DEL "MOSAICO" O "RAYAS AMARILLAS" EN LA CAÑA DE AZUCAR. Rev. Agr., Com. y Trab. [Cuba] 5(1):11-22, illus.
- (12) CHARDON, C. E., and VEVE, R. A.
1922. SOBRE LA TRANSMISIÓN DEL MATIZADO DE LA CAÑA PER MEDIO DE INSECTOS. Rev. Agr. Porto Rico 9:9-20, illus.
- (13) ———
1923. THE TRANSMISSION OF SUGAR-CANE MOSAIC BY APHIS MAIDIS UNDER FIELD CONDITIONS IN PORTO RICO. Phytopathology 13:[24]-29, illus.
- (14) COLÓN, E. D.
1918. YELLOW STRIPE OF SUGARCANE. Porto Rico Insular Expt. Sta. Ann. Rpt. 1917-18:66-68.
- (15) DOOLITTLE, S. P.
1920. THE MOSAIC DISEASE OF CUCURBITS. U. S. Dept. Agr. Bul. 879, 69 p., illus.
- (16) EARLE, F. S.
1919. THE YEAR'S EXPERIENCE WITH SUGARCANE MOSAIC OR YELLOW-STRIPE DISEASE. Jour. Dept. Agr. and Labor Porto Rico 3:15-55.
- (17) FAWCETT, G. L.
1924. EL MOSAICO O ENFERMEDAD DO LAS RAYAS AMARILLAS DE LA CAÑA. Rev. Indus. y Agr. Tucumán 15:103-111, illus.
- (18) HENDERSON, R. G., and WINGARD, S. A.
1931. FURTHER STUDIES ON TOBACCO RING SPOT IN VIRGINIA. Jour. Agr. Research 43:191-207, illus.
- (19) [KAMERLING, Z.]
1903. DE GELE STREPENZIEKTE DER BLADEREN. Proefsta. Suikerriet West Java "Kagok" to Pekalongan, Verslag 1902:76-81.
- (20) KOBUS, J. D.
1908. VERGELIJKENDE PROEVEN OMTRENT GELE-STREPENZIEKTE. Arch. Java-Suikerindus. 16:350-374. Reprinted as Meded. Proefsta. Java-Suikerindus., No. 12, p. [319]-342.
- (21) KUNKEL, L. O.
1921. A POSSIBLE CAUSATIVE AGENT FOR THE MOSAIC DISEASE OF CORN. Hawaii Sugar Planters' Assoc. Expt. Sta. Bul., Bot. Ser. 3:44-58, illus.
- (22) ———
1922. INSECT TRANSMISSION OF YELLOW STRIPE DISEASE. Hawaii. Planters' Rec. 26: 58-64, illus.
- (23) ———
1924. FURTHER STUDIES ON THE INTRACELLULAR BODIES ASSOCIATED WITH CERTAIN MOSAIC DISEASES. Hawaii. Sugar Planters' Assoc. Expt. Sta. Bul., Bot. Ser. 3:108-114, illus.
- (24) LEDEBOER, F.
1921. GELESTREPENZIEKTE. Arch. Suikerindus. Nederland.-Indië, 29 (deel 2):1000-1001.

- (25) LYON, H. L.
1914. LAHAINA CANE INJURED BY YELLOW STRIPE DISEASE. Hawaii. Planters' Rec. 10:320-323.
- (26) ———
1921. THREE MAJOR CANE DISEASES: MOSAIC, SEREH, AND FIJI DISEASE. Hawaii Sugar Planters' Assoc. Expt. Sta. Bul., Bot. Ser. 3:1-43, illus.
- (27) McCLEAN, A. P. D.
1928. MOSAIC DISEASE OF SUGAR CANE. WITH SPECIAL REFERENCE TO ITS ERADICATION IN NATAL. So. African Sugar Jour. 12:483, 485, 487, 489.
- (28) McKINNEY, H. H.
1925. A MOSAIC DISEASE OF WINTER WHEAT AND WINTER RYE. U. S. Dept. Agr. Bul. 1361, 11 p., illus.
- (29) McRAE, W., and SUBRAMANIAM, L. S.
1928. A FURTHER NOTE ON THE MOSAIC DISEASE OF SUGARCANE. Agr. Jour. India 23:239-255, illus.
- (30) MATZ, J.
1919. INFECTION AND NATURE OF YELLOW-STRIPED DISEASE OF CANE (MOSAIC, MOTTLING, ETC.) Jour. Dept. Agr. and Labor Porto Rico 3(4):65-82, illus.
- (31) MUSSCHENBROEK, S. C. VAN.
1898. BESCHRIJVING VAN TWEE TOT DUSVERRE IN WEST-JAVA ONBEKENDE RIETZIEKTEN. SOERABAIASCHE VEREENIGING VAN SUIKER FABRIKANTEN. Circulaire No. 42. Bijlage Arch. Java Suikerindus. 1893 : [113]-118.
- (32) SEIN, F. JR.
1930. A NEW MECHANICAL METHOD FOR ARTIFICIALLY TRANSMITTING SUGAR CANE MOSAIC. Jour. Dept. Agr. Porto Rico 14:49-68.
- (33) STAHL, C. F.
1927. CORN STRIPE DISEASE IN CUBA NOT IDENTICAL WITH SUGAR CANE MOSAIC. Trop. Plant Research Found. Bul. 7, 12 p., illus.
34. ——— and FARIS, J. A.
1929. THE BEHAVIOR OF THE NEW P. O. J. CANES IN RELATION TO SUGAR CANE MOSAIC IN CUBA. Trop. Plant Research Found. Bul. 9, 12 p., illus.
- 35 STEVENSON, J. A.
1917. AN EPIPHYTIC OF CANE DISEASE IN PORTO RICO. Phytopathology 7:[418]-425, illus.
36. ———
1917. REPORT OF THE PATHOLOGIST. Bd. Comm. Agr. Porto Rico Rpt. 5:58-70.
37. ———
1919. THE MOTTLING OR YELLOW-STRIPED DISEASE OF SUGARCANE. Jour. Dept. Agr. and Labor Porto Rico v. 4 (i. e., v. 3 No. 3) No. 1, p. 3-76, illus.
- (38) STOK, J. E. VAN DER.
1907. VERSCHIJNSELEN VAN TUSSCHENRASVARIABILITEIT BIJ HET SUIKERRIET. Meded. Profesta. Oost-Java, ser. 4, No. 36, p. [457]-477.
- (39) WALKER, N. N., and STAHL, C. F.
1926. CERTAIN GRASS HOSTS OF THE SUGAR CANE MOSAIC DISEASE AND OF THE CORN APHID CONSIDERED IN RELATION TO THEIR OCCURRENCE IN CUBA. Trop. Plant Research Found. Bul. 5, 14 p.
- (40) WILBRINK, G.
1922. EEN ONDERZOEK NAAR DE VERBREIDING DER GELESTREPENZIEKTE DOOR BLADLUIZEN. Arch. Suikerindus. Nederland. Indië 30 (deel 2):[413]-456.
- (41) ———
1930. MECHANICAL TRANSMISSION OF SUGAR CANE MOSAIC. Cong. Internatl. Soc. Sugar Cane Technologists. Proc. (1929) 3:155-161.
- (42) ——— and LEDEBOER, F.
1910. BIJDRAGE TOT DE KENNIS DER GELE STREPENZIEKTE. Meded. Proefsta. Java-Suikerindus. deel 2, No. 39, p. 443-495, illus.

VITA

The writer was born August 20, 1886, near Kovna, Lithuania, and his early boyhood was spent at home in an agricultural community on the wooded banks of the river Niemen. Early schooling was of a private character, including the writing and reading of three languages—Russian, German, and Hebrew—, exercises in arithmetic and Bible study. Between the ages of ten and eighteen he attended private schools away from home and the studies were mainly in literature, philosophy and traditional learning. In 1905 he came to Boston, Massachusetts, entering the Massachusetts Agricultural College, at Amherst, in 1909. Most attention was given to the biological sciences, notably entomology, mycology, bacteriology and plant pathology. He was student assistant in botany during his senior year. He was specially influenced by the remarkably clear teaching of Dr. Henry T. Fernald, Professor of Entomology and by friendly encouragement toward research work received from Dr. George E. Stone, Professor of Botany. After receiving the B.Sc. degree in 1913 the writer became assistant in Plant Pathology at the Florida Experiment Station, where he described a new *Rhizoctonia* causing fig leaf blight and studied various other diseases, especially the die-back of the pecan. In 1918, as agent of the U. S. Bureau of Plant Industry, he was engaged in a study of varietal susceptibility of citrus species to *Bacterium citri*. From 1918 to 1922 he had charge of the Department of Botany and Plant Pathology in the Experiment Station at Rio Piedras, Porto Rico, where he discovered the gumming disease and the dry-top rot of sugarcane and studied sugarcane mosaic, among other things. From 1922 to 1929 he was in charge of agricultural research, in Porto Rico and Santo Domingo, for three American sugar-producing companies. He entered the Johns Hopkins University in October, 1929, devoting himself to plant physiology. Since 1930 he has been pathologist in the Office of Sugar Plant Investigations, U. S. Department of Agriculture.

